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RELATED PCT APPLICATION NUMBER: *PCT/US04/11728*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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Certifying Officer

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EU861166867 US

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☐ *Additional inventors are being named on the ___ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

The role of Nogo and NgR in Alzheimer's Disease

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages*	6	☐ CD(s), Number	
☐ Drawing(s) *Number of Sheets*		☐ Other (specify)	
☐ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 25-0110
☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.
☒ Yes, the name of the U.S. Government agency and the Government contract number are: NIH R01 NS 93362 and F31 NS 11007

Respectfully submitted,

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Date 04/16/03

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

NgR in Alzheimer's Disease

We have performed an immunohistologic analysis of Nogo and NgR in human tissues from control and AD sources. There is a clear shift in both the Nogo and NgR protein in the diseased samples (Fig. 1). Nogo is normally prominent in the neuropil of gray matter, but striking pyramidal cell body staining develops in AD. In contrast, the NgR is prominent in pyramidal cell bodies and proximal dendrites of control samples, but cellular staining is greatly decreased in the AD neurons. Coupled with this decrease of neuronal NgR, there is obvious NgR localization in and around amyloid plaques.

To explore the basis of these changes in Nogo and NgR protein in AD, we have examined whether the proteins interact directly with APP or A_. This new line of work has revealed a remarkable finding; NgR physically associates with both APP and A beta. When NgR and APP are overexpressed in the same cell, immunoprecipites of one protein reveal the other protein (Fig. 2). This physical association of NgR and APP results in a shift of a portion of APP protein from intracellular membrane compartments such as the Golgi to the plasma membrane (Fig. 3). Such a shift in subcellular APP localization seems likely alter processing of the protein to disease causing A_. This provides a further rationale for pharmacological manipulation of NgR function in AD.

Since NgR interacts with APP and since NgR accumulates in AD plaques, we considered the possibility that NgR associates with A_ directly. In order to assess this potential interaction, a tagged version of A_ was generated by fusing alkaline phosphatase to human A_ (1-43). This ligand binds tightly to COS cells expressing mouse or human NgR, but not to control COS cells. We also tested if this AP-A_ ligand would bind to purified NgR in an ELISA format. High signal to noise ratios were observed in binding assays demonstrating protein/protein interactions under cell-free conditions.

This data provides one explanation for NgR localization to plaques. It demonstrates that A_ and other NgR ligands interact at the NgR. A_ may act synergistically with myelin proteins to inhibit axon growth through NgR or A_ may block the action of myelin inhibitors and result in axonal sprouting. More importantly, the data show that intervatntions to modulate NgR activity are predicted to alter APP metabolism and Abeta activity.

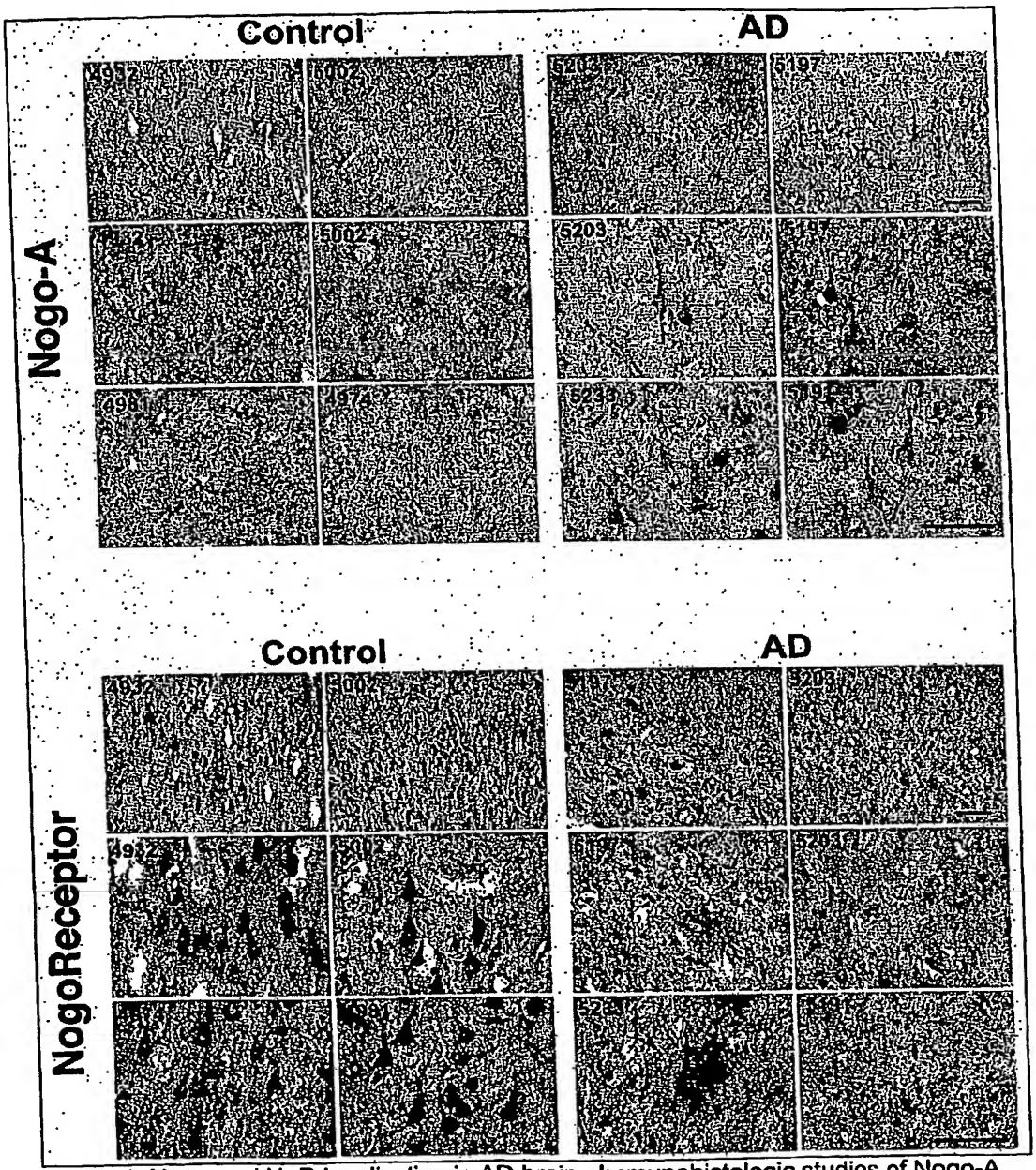

Figure 1. Nogo and NgR localization in AD brain. Immunohistologic studies of Nogo-A or NgR are presented from four different age-matched or AD cases. Note in the top panel that Nogo expression in pyramidal neurons of AD cerebral cortex. In the bottom panel, note that NgR expression is decreased in pyramidal cell bodies but increased in and around some plaques in the AD samples. Similar results were obtained in hippocampal sections. The top row of panels in each half is photographed at lower magnification than the bottom two rows of images.

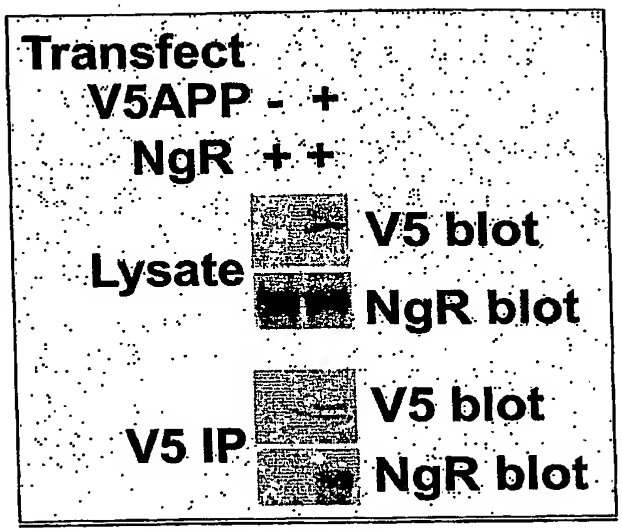

Figure 2. APP and NgR co-immunoprecipitate. HEK 293T cells were transfected with expression vectors for NgR, APP-V5, both or neither. Cell lysates were immunoprecipitated with anti-V5 epitope tag antibodies and then examined by immunoblot. Note that NgR is specifically co-precipitated with APP.

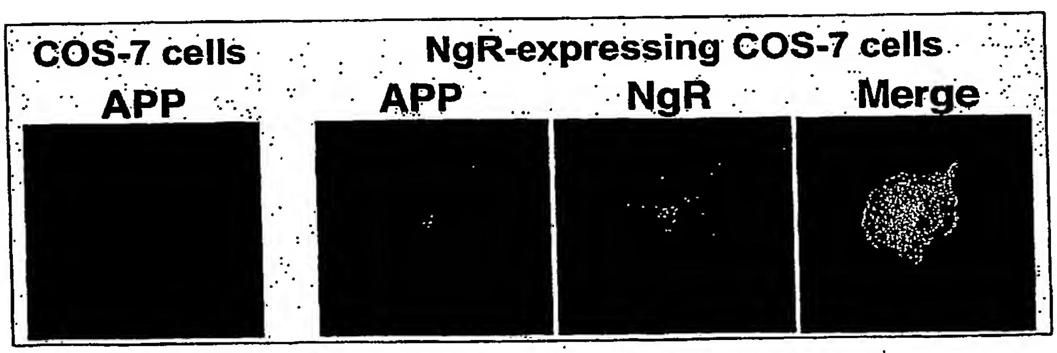

Figure 3. Co-expression of NgR alters APP subcellular localization. The distribution of APP is demonstrated in COS cells with or without NgR co-expression. Note that in cells transfected with the APP expression vector but without NgR, much of the protein is localized intracellularly, and little APP is found on the plasma membrane. In NgR/APP cells, a significant plasma membrane enrichment of APP is detected and that fraction colocalizes with NgR immunoreactivity.

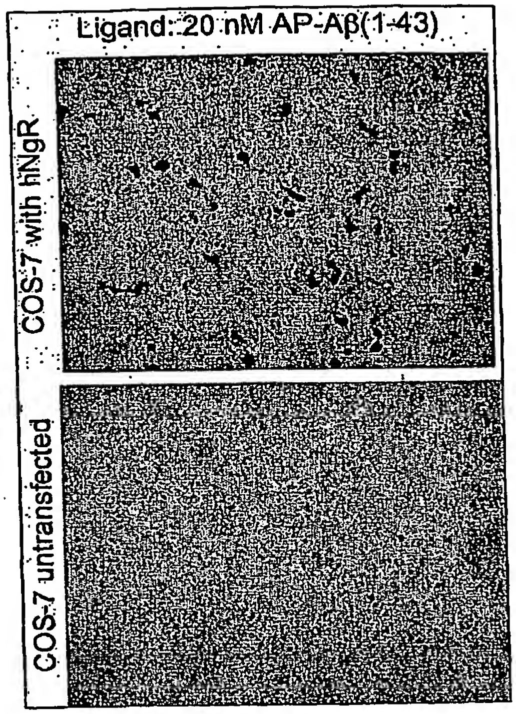

Figure 4. Aβ binds to NgR. The binding of 20 nM fusion protein consisting of Alkaline Phosphatase and Aβ (1-43) to COS-7 cells expressing NgR is illustrated. Note that there is no binding to control non-NgR cells, but prominent binding to cells expressing NgR.

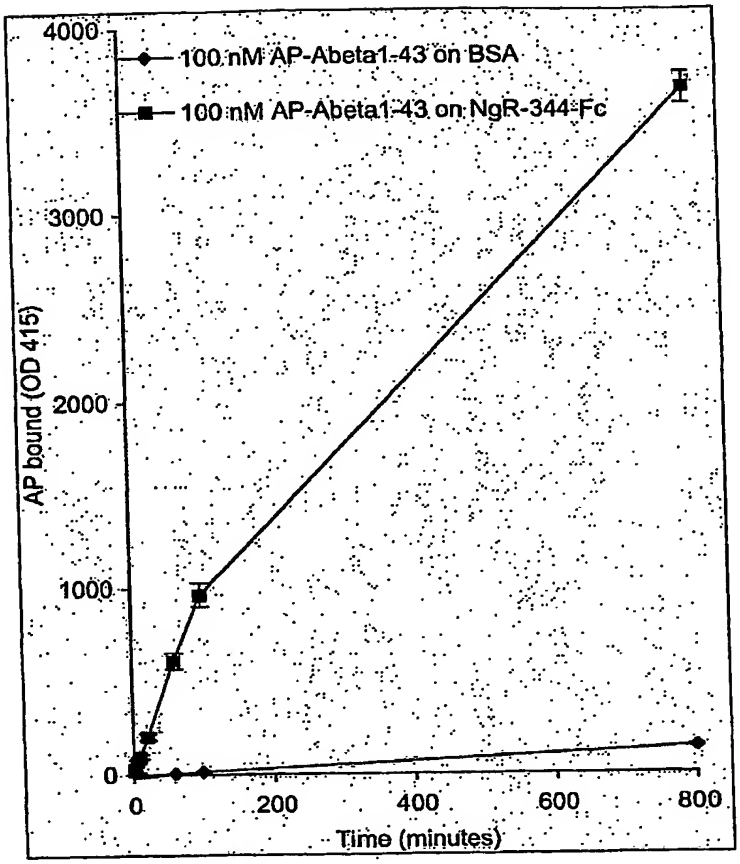

Figure 5. Aβ binds to purified NgR. The binding of 100 nM fusion protein consisting of Alkaline Phosphatase and Aβ (1-43) to immobilized purified NgR is illustrated. Note that there is no binding to control non-NgR wells, but prominent binding to wells coated with NgR.

Methods. I coated Maxisorp Nunc wells with 75 µl of 0.1 mg/ml NgR-344-Fc in 50 mM Tris-HCL, 200 mM NaCl, pH 8.0 for 3 hours at RT. Then I washed with TBS, and blocked all wells with 200 µl of 6% BSA in 50 mM Tris-HCL, 200 mM NaCl, pH 8.0 for 3 hours at RT. After washing with TBS, I applied 75 µl of various concentrations of filtered AP ligand either in COS cell medium + 25 mM NaHepes pH 7.0 or in 1% BSA, 50 NaHepes, 200 mM NaCl, pH 7.0. After 2 hours at RT, I washed 6 times quickly with ice cold TBS and then developed with pNPP at room temp.